

# Large deuterium isotope effects and their use: a historical review

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Isotope effects are differences in the properties of the isotopes of an element resulting in different reaction rates of a corresponding compound, in equilibrium constants and in the spectra. Shortly after the discovery of stable isotopes of hydrogen, oxygen, and carbon, Jacob Bigeleisen formulated a theory of isotope effects and calculated possible maximum values. Large isotope effects of  $^2\text{H}$  (deuterium) against  $^1\text{H}$  (protium) were seen to possibly influence interpretations of reaction mechanisms if corresponding labelling is used. Much work was invested to ensure the safety of deuterium use in men in spite of the large isotope effect. On the other hand, large deuterium isotope effects gave rise to several practical applications. Examples are the enhancement of the stability of some technical products against oxidative and against hydrolytic degradation (oils, pharmaceuticals) as well as alterations of the detoxification metabolism of pharmaceuticals *in vivo*.

**Keywords:** hydrogen-2; isotope application; isotope effects; men; oils; pharmaceuticals; review; safety; stabilisation; technical use

## 1. Introduction

The definition of isotope effects reads as follows:

Isotope effects are differences in the properties of the isotopes of an element as well as the resulting effects [1], and, in more detail:

Isotope effects are effects on the rate (kinetic isotope effects) or equilibrium constants (thermodynamic isotope effects) of two systems that differ only in the isotopic composition of one or more of their otherwise chemically identical components. Commonly, the value of a kinetic isotope effect is given as the quotient of the two rate constants  $k_1/k_2$ .

Isotope effects were the pre-condition for the discovery of stable isotopes in the 1930s. Isotope effects are the basis of all isotope analyses and of all production ways, which are mostly methods to enrich the less frequent heavier isotope against its natural abundance.

As is well known, the different nuclear masses of the isotopes of an element are the main cause of isotope effects; however, nuclear volume and nuclear magnetism also contribute to certain isotope effects, for example in optical, NMR, and ESR spectra.

In principle, an isotope effect should be the larger, the larger the mass difference between the isotopes is. The heavy stable isotope of the lightest element hydrogen,  $^2\text{H}$  (deuterium), has the highest mass difference against its light isotope,  $^1\text{H}$  (protium), namely the double mass of  $^1\text{H}$ .

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Table 1. Calculated maximum values of kinetic isotope effects for the case of a bond-free transition state at 25 °C [18], adapted from [2].

Isotope 1	Isotope 2	$k_1/k_2$
$^1\text{H}$	$^2\text{H}$	18
$^1\text{H}$	$^3\text{H}$	60
$^{10}\text{B}$	$^{11}\text{B}$	1.3
$^{12}\text{C}$	$^{13}\text{C}$	1.25
$^{12}\text{C}$	$^{14}\text{C}$	1.5
$^{14}\text{N}$	$^{15}\text{N}$	1.14
$^{16}\text{O}$	$^{18}\text{O}$	1.19
$^{24}\text{Mg}$	$^{27}\text{Mg}$	1.08
$^{32}\text{S}$	$^{35}\text{S}$	1.05
$^{40}\text{Ca}$	$^{45}\text{Ca}$	1.08

Therefore, the isotope effect between  $^3\text{H}$  (tritium) and  $^1\text{H}$  should reach the highest thinkable value. In this review, the radioactive isotope  $^3\text{H}$  will not be considered.

Starting already in the 1940s, Jacob Bigeleisen was the most important pioneer in the field of theory of isotope effects (see obituary in *Isot. Environ. Health Stud.* 46, 403 (2010)).

Bigeleisen used the moments of inertia and the masses of the isotope pairs of a given element and assumed a bond-free transition state during a bond splitting to calculate the theoretical maximum values of reaction kinetic isotope effects [2] (Table 1).

Apparently, the reaction kinetic isotope effects of isotope pairs of all elements heavier than hydrogen are very small compared with those of the hydrogen isotopes. According to Bigeleisen, the theoretical maximum value of the reaction kinetic isotope effect of the isotope pair  $^1\text{H}/^2\text{H}$  is 18. That means, in simple words, that a reaction with a rate-limiting step of a  $\text{C}-^2\text{H}$  bond splitting may have an up to 18-fold slower reaction rate compared with a  $\text{C}-^1\text{H}$  bond. What about the consequences concerning

- errors in labelling studies,
- thinkable poison at labelling studies in plants, animals, and even humans,
- thinkable practical applications?

What about the deuterium isotope effects in more complicated reactions such as radical reactions and enzymatically controlled reactions?

Up to the present, there are a lot of studies about isotope effects and many corresponding books [3–9]. However, studies on very large deuterium isotope effects are rare. This is also true for studies on possible damage to plants, animals, and even humans by deuterium application, and on the technical or pharmacological application of large isotope effects. Corresponding studies had their height in the 1960s and 1970s. A centre of research in this field was the Leipzig Institute for Stable Isotopes (1956–1990), at that time in East Germany (the former GDR). Therefore, a lot of those studies were published in German, partly in East Germany and in works not commonly reachable (e.g. proceedings of meetings in East Europe, periodical reports on the work of scientific institutions, such as ‘ZfI-Mitteilungen’).

It is the aim of this paper to review studies about very large deuterium isotope effects and about their practical application, and to use the opportunity also to return to mind some of the studies, which were not easily accessible to interested scientists.

## 2. Approval of studies with stable isotopes in humans

Are enriched stable isotopes universally allowed to be used in humans? More specifically, the question has to be answered: Is there a potential risk if a substance with unnaturally changed

isotope ratios is administered to the human body? As is known, the stable isotopes of the bioelements hydrogen, carbon, oxygen, and nitrogen show a certain variation within relatively narrow limits in nature. If, however, the ratio is changed drastically, say, from 0.0147 at% natural  $^2\text{H}$  abundance to 99 at%  $^2\text{H}$  (versus 1 at%  $^1\text{H}$ , correspondingly), what about the high theoretical isotope effect to be considered *in vivo*? Is there any poison if the isotope ratio also of heavier bioelements is changed drastically, in spite of the relatively small isotope effects calculated by Bigeleisen?

Already in 1936, Erlenmeyer measured a considerably slower reaction rate for the enzymatic oxidation of deuterated succinate compared with non-labelled succinate *in vitro* [10].

In 1963 and 1964, first papers were published about biological effects of deuterium [11] and, more generally, about effects of the nuclear mass on biological systems [12].

Phenomena were discussed such as slower growth rates and slower cell division in simple unicellular organisms but also heavy disorganisations of the metabolic combination in more complex systems such as animals.

For example, Hughes *et al.* [13] showed that 30%  $^2\text{H}_2\text{O}$  in the drinking water of mice inhibit their spermatogenesis. Fish do not survive in water with 30%  $^2\text{H}_2\text{O}$ . Apparently, several deuterium isotope effects contribute to this phenomenon.

As a consequence of various deuterium tests with animals, the high deuterium isotope effects must be considered if deuterium-labelled compounds are applied to men. Special attention must be paid to the compound heavy water ( $^2\text{H}_2\text{O}$ ). Its influences on biochemical reactions *in vivo* are characterised mainly with the term solvent isotope effect. Besides the different molecular weight (20.028 against 18.015), all the other physical properties of  $^2\text{H}_2\text{O}$  are also different from the given values of  $\text{H}_2\text{O}$  with the natural isotope abundances. Some properties are compared in Table 2 [1]. Some of the properties of  $^2\text{H}_2\text{O}$  may contribute to adverse health effects.

Due to the considerably smaller isotope effects expected for elements heavier than hydrogen, corresponding health effects should be very small, not measurable or even negligible and insignificant. This hypothesis is supported by some studies with animals, too.

Mice were administered  $^{18}\text{O}_2$  via breath and  $\text{H}_2^{18}\text{O}$  via drinking water up to a level of 60 at%  $^{18}\text{O}$  over 112 days. As a result, no histological and ontogenetic aberrations over three generations occurred [14]. On the other hand, in an earlier study, anomalies in the growth of microorganisms were seen after  $^{18}\text{O}$  application [15]. Not to risk any fatal use and to be on the safe side,  $\text{H}_2^{18}\text{O}$  was placed, together with  $^2\text{H}_2\text{O}$ , into a category of toxic compounds.

Table 2. Physical properties of  $^2\text{H}_2\text{O}$  compared with water with the natural isotope abundances [1].

	$^2\text{H}_2\text{O}$	Natural water
Molecular mass ( $\text{g mol}^{-1}$ )	20.028233	18.015585
Freezing temperature ( $^{\circ}\text{C}$ at 1 bar)	3.813	0.00
Melting heat ( $\text{kJ mol}^{-1}$ at 1 bar)	6.284	6.012
Boiling point ( $^{\circ}\text{C}$ at 1 bar)	101.43	100.00
Vapour pressure (Torr at $20^{\circ}\text{C}$ )	15.24	17.54
Vapour pressure (Torr at $100^{\circ}\text{C}$ )	721.6	760.0
Critical temperature ( $^{\circ}\text{C}$ )	371.5	374.2
Temperature of the max. density ( $^{\circ}\text{C}$ , 1 bar)	11.23	3.98
Density ( $\text{g cm}^{-3}$ at $20^{\circ}\text{C}$ )	1.10536	0.998232
Viscosity (millipoise at $20^{\circ}\text{C}$ )	12.60	10.05
Solubility of NaCl ( $\text{g}/100\text{ g}$ at $25^{\circ}\text{C}$ )	30.56	35.92
Electrical conductivity of a 0.01 molar KCl solution ( $\Omega^{-1}\text{ cm}^2$ at $25^{\circ}\text{C}$ )	117.0	141.3
Ion motility in aqueous solution ( $25^{\circ}\text{C}$ )		
$^2\text{H}_3\text{O}^{+}$	250.1	
$\text{H}_3\text{O}^{+}$		349.8

For the isotope pair  $^{12}\text{C}/^{13}\text{C}$ , Bigeleisen had calculated a theoretical maximum value of  $k_1/k_2 = 1.25$ , and for  $^{14}\text{N}/^{15}\text{N} = 1.14$ . Experimental values are generally smaller. Comprehensive investigations aimed to search for biological effects of high  $^{13}\text{C}$  loads on 271 mice embryos. No changes in the normal development were found that could have been caused by the unnatural isotope ratio [16].

By analogy, the influence of a high  $^{15}\text{N}$  enrichment on life processes can be claimed to be negligibly small – even though comparable investigations over generations of animals are not known. For enzymatic reactions, the measured  $^{14}\text{N}/^{15}\text{N}$  isotope effects were as small as 1.010 (arginine hydrolysis) and 1.008 (urea hydrolysis) [17].

Preparing any administration of stable isotopes with unnatural abundances to the human body, not only the value of an isotope effect measured earlier must be considered but furthermore:

- the dose and isotope enrichment which reach ‘critical organs’ as well as
- the incorporation tendency of the enriched isotope under discussion in the critical organ.

The most serious conceivable accident could happen if a high dose of a highly bioactive substance (or its precursor) highly enriched with  $^2\text{H}$  was injected, e.g. into fast dividing cells. Here, the three conditions mentioned above (maximum relative mass difference, high dose and enrichment as well as strong incorporation tendency) would be fulfilled. This theoretical situation is not encountered in practical use [18,19].

Generally, economic considerations are strong arguments that make the investor keep the dose (and frequent repetitions of a test with the same subject) as small as reasonable.

Then the applied dose is quickly distributed in the body and therefore – in the case of labelled water – diluted. As a result, the enrichment of the tracer isotope decreases and is no longer far away from the natural isotope abundance.

A special case of an influence of artificially changed isotope ratios to be considered is that in combination with ionising radiation. On this occasion, isotopes can be activated and may undergo a nuclear reaction yielding radioactive nuclides. In this context, only  $^{10}\text{B}$  is of interest, if used in the medical neutron capture therapy due to its enhanced neutron capture cross-section [20].

Summarising the results of numerous investigations to estimate the application risks of enriched stable isotopes in men, the following conclusion can be drawn:

There is some potential risk in the clinical use of  $^2\text{H}$ , of  $^{18}\text{O}$  (as  $\text{H}_2^{18}\text{O}$  only), and of  $^{10}\text{B}$  considering its enhanced neutron capture capacity.

Enriched isotopes of elements heavier than hydrogen – with the above-mentioned exceptions – are not known to interfere in the normal cell. This is not an absolute statement and only today’s state of knowledge. If a user has any doubt, he should be cautious and do further experiments before labelling a man. His decision should be clear before an Ethical Committee is confronted with the project.

Nowadays, tests with  $^2\text{H}_2\text{O}$  and with  $^2\text{H}_2^{18}\text{O}$  are in clinical and epidemiological routine use, for example promoted by the IAEA [21]. The amounts of labelled water used in the tests are small.

An astonishing fact is that – to our knowledge – only one state regulation was passed, namely already in 1969. To negotiate insecurities in the human application of stable isotopes, a group of competent East German scientists had searched through the international literature in order to look for studies which show possible borderlines where stable isotope use poses a risk to human health. As a result, an official human health standard was published in the former GDR: ‘Stable Isotope Labelled Chemical Elements and Compounds. Part 3: Application in Human Medicine and Food Industry’ [22].

The main rules of the standard were:

*Statutory consents:*

- Elements with a relative atom mass larger than 20 have so small isotope effects that no health threat can occur. The application of chemical compounds containing stable isotope-labelled elements with a relative atom mass larger than 20 is admissible without restrictions.
- The application of chemical compounds containing the stable isotope-labelled elements carbon or nitrogen is admissible without restrictions.
- The application of chemical compounds – with the exception of water – containing the stable isotope-labelled element oxygen is admissible without restrictions.
- The application of chemical compounds – with the exception of water – containing the element hydrogen which is labelled with deuterium up to 15 at% is admissible without restrictions.
- Chemical compounds containing the stable isotope-labelled elements carbon, nitrogen, oxygen – with the exception of water – or elements with a relative atom mass larger than 20 are not classified as modified substantially if used in food industry.

*Limitations:*

- The application of chemical compounds containing the element hydrogen which is labelled with deuterium to more than 15 at% may be used in medicine only after a pharmacological test mandatory by the state laws.
- Water labelled with deuterium is permitted to be administered in only such an amount that the mean deuterium abundance in the body water does not exceed 0.6 at%.
- Water labelled with  $^{18}\text{O}$  is permitted to be administered in only such an amount that the mean  $^{18}\text{O}$  abundance in the body water does not exceed 5 at%.
- The insertion of deuterium- or  $^{18}\text{O}$ -labelled water into organs with fast cell division is permitted only after a pharmacological test mandatory by the state laws.
- Chemical compounds containing deuterium-labelled hydrogen are not allowed to be used in food industry.
- Water labelled with more than 0.5 at% deuterium or with more than 2.0 at%  $^{18}\text{O}$  is not allowed to be used as drinking water.

Of course, these provisions are not binding internationally. They were given as official regulations at that time in that country. However, for lack of modern international regulations they can be used as a guideline.

Suppliers of stable isotope-labelled compounds often inform the purchaser that the offered products are intended for laboratory or manufactory use and not made for human consumption. The verification of their suitability for human or animal use as clinical diagnostic or therapeutic agents is claimed to be the sole responsibility of the user. For example, a company claims: 'Approval from the appropriate government regulatory agency should be obtained before the researcher begins any studies in which he plans to administer our stable isotope labelled compounds to humans' [23].

To our knowledge, no actual rules have become available since our corresponding search in 1991 [18,24].

In 1999, Kushner *et al.* [25] reviewed the state of scientific research in pharmacology as follows:

Experiments in mice, rats, and dogs have shown that a degree of 25% deuteration causes (sometimes irreversible) sterility, because neither gametes nor zygotes can develop. High concentrations of heavy water (90%) rapidly kill fish, tadpoles, flatworms, and *Drosophila*. Mammals, such as rats, given heavy water to drink die after a week, at a time when their body water approaches about 50% deuteration. The mode of death appears to be the same as that in cytotoxic poisoning (such as chemotherapy) or in acute radiation syndrome (though deuterium is not radioactive), and is due to deuterium's action in generally inhibiting cell division. It is more toxic to malignant cells than normal cells but the concentrations needed are too high for regular use. As in chemotherapy, deuterium-poisoned mammals die of a failure of bone marrow (bleeding and infection) and intestinal-barrier functions (diarrhea and fluid loss). . .  $\text{D}_2\text{O}$

is more toxic to malignant than normal animal cells . . . Protozoa are able to withstand up to 70% D<sub>2</sub>O. Algae and bacteria can adapt to grow in 100% D<sub>2</sub>O.

Kushner also reviewed the generally recognised codes of deuterium use in humans. He ascertained that oral doses of several grams of heavy water (and of <sup>18</sup>O) are routinely used in the assessment of body composition and total energy expenditure in humans.

A 50 kg human body containing 32 kg of body water normally contains about 1.1 g deuterium equal to 5.5 g of pure heavy water, so roughly such a dose is required only to double the amount of deuterium in the body.

According to Kushner, full replacement with isotopes of heavier atoms (such as carbon-13, nitrogen-15, and oxygen-18) can be accomplished in higher organisms, but this cannot be done for deuterium. In cancer therapy, deuterium oxide is used to enhance boron neutron capture, but this effect does not rely on the biological effects of deuterium per se [25].

### 3. Consideration of isotope effects in labelling studies

A general assumption of any tracer experiment with stable isotopes is that in the system under investigation, the behaviour of the labelled substance (tracer) is identical to that of the unlabelled substance (tracee). Herewith, the benefit of stable isotopes is used to follow a chemical or biochemical reaction *in vitro* or *in vivo* step by step – which is not feasible with non-isotopic labelling as with most of radioactive tracers (e.g. <sup>99m</sup>Tc).

Unfortunately, there are isotope effects which may disturb this assumption. Fortunately, with the exception of the isotope pair protium/deuterium, isotope effects are small. They are commonly ignored in tracer experiments with <sup>13</sup>C, <sup>15</sup>N, or <sup>18</sup>O as labelling tracers. Deuterium is used as a tracer only if an alternative labelling with isotopes of one of the heavier elements is not feasible.

In tracer studies with deuterium, the isotope effect on the reaction rate should be considered and estimated separately [26].

There is another disadvantage of deuterium as a tracer in chemistry and biochemistry. It is the intramolecular and intermolecular isotope exchange of the hydrogen isotopes up to a certain equilibrium depending on the character of the H bond and of the medium. The phenomenon was reviewed by Schattenstein [27] considering the literature up to 1960 (1074 references).

Both facts are handicaps and often make deuterium unsuited for labelling studies.

### 4. Practical use of deuterium isotope effects

#### 4.1. Use to enrich deuterium

The well-known heavy water (<sup>2</sup>H<sub>2</sub>O) has been the first and up to now the most important technical product of deuterium. The principal peaceful historical demand for deuterium oxide has been its use in certain nuclear reactors (heavy water reactors) as an excellent neutron captor because of its low absorption cross-section for slow neutrons. A nuclear reactor moderated and cooled with <sup>2</sup>H<sub>2</sub>O can be fuelled with natural uranium thus avoiding <sup>235</sup>U enrichment. In the future, heavy water will be used in breeder reactors, too.

Primarily, deuterium was manufactured as a by-product in a plant installed by Norsk Hydro in Norway in 1934, originally to produce hydrogen. The plant operated on the basis of water electrolysis due to the high separation factor, however, with high energy cost. In 1940, the plant came temporarily under the control of Germany. Since the 1940s, large-scale production plants

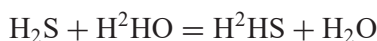
Table 3. Separation factors for  $^2\text{H}$  enrichment methods.

Method	$k_{\text{H}}/k_{\text{D}}$
Chemical exchange	3
Distillation	1.05–1.7
Gaseous diffusion	1.2
Centrifugation	1.01–1.11

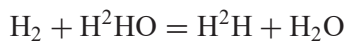
were installed in several further countries and with different methods. In those times, heavy water gained notoriety as a feed material for nuclear weapons.

Separation factors as a technical term of corresponding isotope effects have been compiled for different methods by van Hook [28] (Table 3).

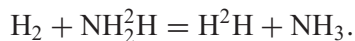
Considering energy cost and capital cost per separation unit, the most promising methods are nowadays those based on chemical exchange such as:



or



or



Because of the very high enrichments required in  $^2\text{H}_2\text{O}$  production, water distillation is favoured in the upper enrichment stages, starting with about 30 at%  $^2\text{H}_2\text{O}$  to reach more than 99.9 at%. Highly enriched  $^2\text{H}_2\text{O}$  is also used as a solvent in NMR spectroscopy and to prepare further deuterium-labelled compounds via isotope exchange or via syntheses.

#### 4.2. Potential use in pharmacology: retarded metabolism and metabolic switching in vivo

The title of a comprehensive review written in 1985 by Foster reads ‘Deuterium isotope effects in the metabolism of drugs and xenobiotics: implications for drug design’ [29]. Evaluating 134 original papers and 10 reviews, Foster focused on the effect of a substitution of deuterium for hydrogen in various C–H bonds. Hydroxylation reaction rates of hydrocarbons with a carbon atom in  $\alpha$  position to oxygen and with a carbon atom in  $\alpha$  position to nitrogen can be very much smaller after deuteration in the corresponding molecule position. As the *in vivo* degradation of a drug is mostly started by hydroxylation (mono-oxygenation), the degradation to a metabolite may be delayed more or less. The organism thereupon avoids this metabolism and tries to eliminate the xenobiotic via another metabolic step (and this with a slower rate).

Already in 1975, Horning *et al.* [30] introduced the term ‘metabolic switching’ for this phenomenon. The deuteration may aim at an extension of the pharmacological effect of the drug (slower metabolism rate) and/or at a decrease in the formation of a more toxic metabolite than the precursor is (switching to another metabolism). As the chemical nature of the drug is not changed by a deuteration, the principal pharmacological effect itself is not changed, either.

Horning *et al.* reviewed a lot of examples for the retardation effect of deuteration. In an own study, they found that after injection of antipyrine trideuterated in the C-3-methyl group to rats, the metabolites in urine were changed. The change from the oxygenation of the C-3-methyl group – the normal major pathway – to N-demethylation (normally the minor pathway) is equal to a large kinetic isotope effect of about 15 [29].

Another example is the prolongation of the half-life of the effective form of the anaesthetic agent butobarbital.  $[3\text{'-}^2\text{H}_2]\text{Butobarbital}$  has a biological half-life which is 2.5 times longer than that of the undeuterated drug [31].

In summary, three groups of pharmaceuticals were investigated aiming at an increase in their pharmacological effect and/or aiming at the suppression of side reactions with toxic metabolites:

- inhalation anaesthetics [32],
- anti-cancer drugs [33], and
- antibiotics [34].

During the 2nd International Symposium on Synthesis and Application of Isotopically Labelled Compounds in 1985, two deuterated pharmaceuticals were selected by American manufacturers for scale-up studies for large-scale production, namely 2-deutero-enflurane ( $[^2\text{H}]\text{CF}_2\text{H-O-CF}_2\text{-CClF}$ ) [33] and D-3-fluoro- $[2\text{'-}^2\text{H}]\text{alanine}$  [35]. On this symposium, however, one of the chairmen (W.A. Garland [36]) discussed economic considerations citing also a comment of Foster on the given situation. The key argument was: The advances of the new drug must significantly outweigh the additional cost associated with the synthesis of the deuterated analogue. Moreover, additional cost will be associated with preclinical toxicology and clinical trials. 'It seems very unlikely that the regulatory authorities associated with the pharmaceutical industry would regard a deuterated drug designed to have a biological activity significantly different from that of the parent protium form as other than a new drug' [37].

To our knowledge, the priority of business over technical progress has controlled the decision about deuterated drugs also up to now.

Over the past decades, several production ways for deuterated organic compounds with names mostly sounding like precursors in pharmacy and pharmacology have been patented. Their economical intent has not been disclosed.

#### 4.3. *Use of $^2\text{H}_2\text{O}$ solvent isotope effects to stabilise chemical compounds*

As elucidated above (Chapter 2), the solvent isotope effect of  $^2\text{H}_2\text{O}$  must be kept in mind if it is applied to living species, first and foremost to humans. On the other hand, the solvent isotope effect can be beneficial to stabilise a technical product which is decomposed too quickly in an aqueous solution.

The diagnostic dyestuff indocyanine green (Cardio Green®) is quickly hydrolysed in aqueous solution as an injection agent to investigate blood flow in the liver. From 1965 onwards, our Leipzig group did some fundamental studies in order to enhance the stability of aqueous solutions of interest [38]. For the first time, we were able to show that indocyanine green is more stable in  $^2\text{H}_2\text{O}$  and in  $[1,2\text{'-}^2\text{H}_2]\text{propandiol}$  than in the non-deuterated solvents by a factor of 1.5 [39] (Figure 1). For the procedure, a patent 'Stabilised aqueous, injectable solutions of indocyanine dyestuffs' had been issued in several countries [40].

A similar solvent isotope effect was observed by our group with the anti-cancer agent cyclophosphamide [18, p. 107].

Later on, in 1995, a paper 'Thermostabilisation of live virus vaccines by heavy water ( $\text{D}_2\text{O}$ )' [41] was published by another group without mentioning the earlier studies.

In 1993, the principle of  $^2\text{H}_2\text{O}$  use is seen to be proposed for therapeutic purpose in human medicine. In a corresponding patent, methods of treatment of hypertension using  $^2\text{H}_2\text{O}$ , deuterated foods or deuterated antihypertensive drugs are claimed, later specified by several deuterated drugs. A therapeutically effective amount of  $^2\text{H}_2\text{O}$  is proposed 'in the range of about 10 weight percent to about 90 weight percent based on the weight of water for a time period sufficient to effect a

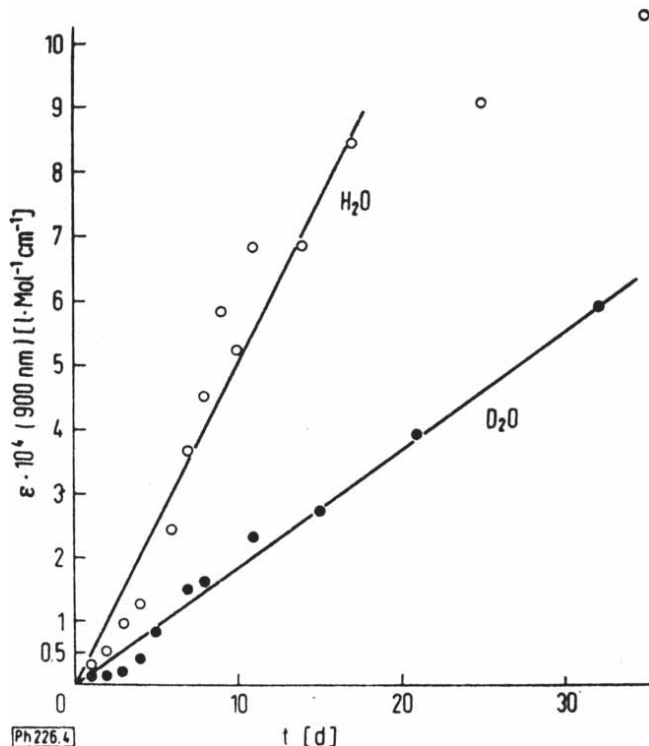


Figure 1. Temporal intensity increase of the absorption maximum at 900 nm of  $10^{-4}$  molar  $\text{H}_2\text{O}$  and  $^2\text{H}_2\text{O}$  solutions of Indocyanine Green as a measure of its age stability [39].

reduction of said blood pressure in a patient'. [42]. Here, discrepancies must be seen to the rules cited above which advise more caution.

#### 4.4. The history of quality improvement of technical goods and devices by deuterium substitution

Many technical goods (lubricants, plastics, and other synthetic materials) are not stable enough over a longer time because of ageing by autoxidation processes.

It was the proposition of Justus Mühlenpfordt, former director of the Leipzig Institute of Stable Isotopes, to start with research to disclose large kinetic deuterium isotope effects in the late 1950s. Hoping for detectable or even high effects, our Leipzig team from then on aimed for a potential use of kinetic deuterium isotope effects to stabilise technical goods and thus improve their quality. In certain molecule groups of organic compounds that are subject to oxidation, deuterium should be substituted for hydrogen.

In a first comprehensive study, xylenes were used as model substances. The xylene isomers were deuterated in the methyl groups by isotope exchange with  $^2\text{H}_2\text{O}$ . Deuterated and undeuterated xylenes were exposed to oxygen at different temperatures, and the oxidation rate was measured continuously. The first corresponding publication had the title 'Kinetic investigations on the catalytic oxidation of xylenes by labelling with deuterium' [43]. The isotope effect of the oxidation of *m*-[dimethyl- $^2\text{H}_6$ ]xylene was seen to be as large as  $k_{\text{H}}/k_{\text{D}} = 16.6$  at  $100^\circ\text{C}$ , a value as high as never measured before.

To our knowledge, this was the first study about very large kinetic isotope effects of deuterium. These isotope effects were also discussed with Jacob Bigeleisen who had calculated a maximum value of  $k_{\text{H}}/k_{\text{D}} = 18$  for a one-step reaction splitting of a  $\text{C}-^2\text{H}$  bond. Our experimental values, however, are the result of several isotope effects summarised over a chain reaction.

Mühlenpfordt decided to continue the research and started a series of publications with the main theme ‘Improvement of industrial goods by substitution of deuterium for hydrogen’ [44].

By the way, it should be mentioned that some of the first papers of the series were published in this journal, at that time named ‘Isotopenpraxis’.

The first study suggesting a technical application of a kinetic deuterium isotope effect was already published in 1965 in the proceedings of the 7th Symposium on Lubricants and Lubrication Technique in Dresden, East Germany, and in more detail in a corresponding journal [45]. Under the title ‘Deuterium substitution as a new method to finish lubricants’, the authors had made deuterated white oil from normal technical white oil by isotope exchange with  $^2\text{H}_2\text{SO}_4$  and with gaseous  $^2\text{H}_2$ . Before oxidation runs, the undeuterated white oils were treated in the same way with  $\text{H}_2\text{SO}_4$  or with  $\text{H}_2$ . The mean of repeated oxidation runs at 110 °C and 120 °C showed an isotope effect of  $k_{\text{H}}/k_{\text{D}} = 2.7\text{--}4.6$ . When the oxidation was inhibited by  $\beta$ -naphthene, the inhibition time was prolonged by a factor of 2.4 (Figure 2).

In modern sophisticated techniques and goods such as special watches, white oil did not meet the demands due to a poor lubricating quality. However, the authors concluded that the results demonstrate further potential to stabilise technical goods by deuterium. According to the theory and already practically seen with the xylenes [43], the isotope effect and, therefore, the profit of the deuteration would be still much higher at room temperature. Application research and the search for application fields were extended.

In the third note of the series founded by Mühlenpfordt, the effect of deuteration on the age stability of a synthetic lubricant was studied. As a model, undeuterated and specifically deuterated succinic di-benzyl esters (SBE) were synthesised with 90–91 at%  $^2\text{H}$  in different positions:

- (a)  $\text{C}_6\text{H}_5\text{--CH}_2\text{OOCCH}_2\text{CH}_2\text{COOCH}_2\text{--C}_6\text{H}_5$  (SBE),
- (b)  $\text{C}_6\text{H}_5\text{--C}^2\text{H}_2\text{OOC}^2\text{H}_2\text{C}^2\text{H}_2\text{COOC}^2\text{H}_2\text{--C}_6\text{H}_5$  (S- $[\text{}^2\text{H}_4]\text{B-}[\alpha,\alpha'\text{}^2\text{H}_4]\text{E}$ ),
- (c)  $\text{C}_6\text{H}_5\text{--C}^2\text{H}_2\text{OOCCH}_2\text{CH}_2\text{COOC}^2\text{H}_2\text{--C}_6\text{H}_5$  (SB- $[\alpha,\alpha'\text{}^2\text{H}_4]\text{E}$ ),
- (d)  $\text{C}_6\text{H}_5\text{--CH}_2\text{OOC}^2\text{H}_2\text{C}^2\text{H}_2\text{COOCH}_2\text{--C}_6\text{H}_5$  (S- $[\text{}^2\text{H}_4]\text{BE}$ ).

At an oxidation temperature of 191 °C compounds (b) and (c) showed isotope effects  $k_{\text{H}}/k_{\text{D}} = 4.3\text{--}4.6 \pm 0.5$  compared with compound (a). Compound (d) showed nearly no isotope effect

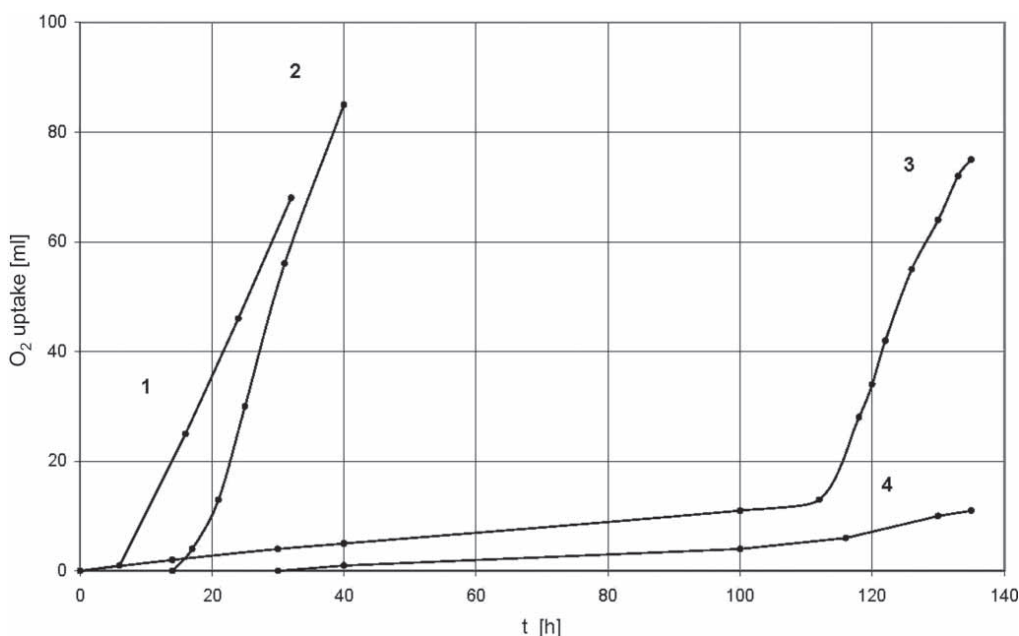


Figure 2. Comparison of oxygen uptake at 100 °C of four watch oils [57]:

1, Classical so-called neatsfoot oil; 2, ether alcohol oil, undeuterated; 3, international top watch oil; 4, Deuteriosynth®.

compared with compound (a). As a consequence, oxygen attacks nearly only on the  $\alpha$ -CH<sub>2</sub> groups of the benzyl side of the molecule. For economic reasons, deuteration is restricted to these positions [46].

As an important component of the application research, careful fundamental research was continued. In a series 'Isotope effects in autoxidations', dependences of the measured quantity of an isotope effect on the constitution and the properties of the chemical compound were studied and published in a series of papers with Krumbiegel [43] being the first one [47–50].

To maximise an expected deuterium isotope effect, several facts, such as specifics of chain radical reaction steps during oxygen attack and during thermal degradation, should be considered [48–49].

The largest kinetic deuterium isotope effect measured up to that time had been  $k_H/k_D = 76$  for the autoxidation of cumene catalysed by cobalt stearate at 65 °C [47].

In all the autoxidation studies, special equipments were used to ensure comparable reaction conditions such as constant oxidation temperatures and exact oxygen uptake measurements [50].

In 1968, a preliminary estimate was published: 'Large hydrogen–deuterium isotope effects under the leading point of their use' [51]. Considering reaction types, reaction conditions, and structures of substances, the requirements for reaching the largest possible deuterium isotope effects are discussed. One group of requirements is given by the classic theory such as symmetrical transition state of the reaction, maximum differences of activation energy of the rate-controlling step, and low reaction temperature. The other group compiles those parameters which enlarge the effect by tunnelling, many chain reaction steps, and induced change of the reaction route. Additional contributions come from the influence of inhibitors, catalysts, or other additives.

Fundamental research about large deuterium isotope effects was continued by Rummel and others [52–54].

So-called synthetic ester lubricants have excellent lubrication qualities at deep and high temperatures and low evaporation tendency. Their stability against ageing, however, is poor. In the framework of the Leipzig research project mentioned above, another model reaction was studied, namely the oxidation of adipinic acid di-*n*-amyl esters deuterated in different molecule positions. The isotope effects measured for a cobalt stearate-catalysed oxidation at 135 °C are different in dependence on the position of the deuterium substitution. The effect is relatively large if two CH<sub>2</sub> groups next to the carboxylic group are deuterated (Table 4) [52]. For technical use, the relatively small values seemed to be unattractive.

To continue the search for large isotope effects, the effect of different alkyl groups was studied in a group of benzyl ethers. As oxidation takes place also on the alkyl groups – not deuterated – the largest effect was measured with the compound without additional alkyl groups, C<sub>6</sub>H<sub>5</sub>-C<sup>2</sup>H<sub>2</sub>-O-C<sup>2</sup>H<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>, namely  $k_H/k_D = 40.4$  at 157 °C. Alkyl-substituted [ $\alpha, \alpha', \alpha', \alpha'$ -<sup>2</sup>H<sub>4</sub>]benzyl ethers showed smaller isotope effects, 'diluted' by concurrent oxidation (and oxygen consumption) by non-deuterated alkyl groups [53].

Measurements about the dependence of the value of an isotope effect on the deuterium abundance were undertaken [54].

Table 4. Kinetic isotope effects in the oxidation of adipinic acid di-amyl esters deuterated in different molecule positions [52].

	$k_H/k_D$
(CH <sub>2</sub> ) <sub>4</sub> -(COOH-C <sup>2</sup> H <sub>2</sub> -(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>3</sub> ) <sub>2</sub>	1.59
(CH <sub>2</sub> ) <sub>4</sub> -(COOH-CH <sub>2</sub> -C <sup>2</sup> H <sub>2</sub> -(CH <sub>2</sub> ) <sub>2</sub> -CH <sub>3</sub> ) <sub>2</sub>	1.28
(CH <sub>2</sub> ) <sub>4</sub> -(COOH-(CH <sub>2</sub> ) <sub>2</sub> -C <sup>2</sup> H <sub>2</sub> -CH <sub>2</sub> -CH <sub>3</sub> ) <sub>2</sub>	1.12
(CH <sub>2</sub> ) <sub>4</sub> -(COOH-(C <sup>2</sup> H <sub>2</sub> -C <sup>2</sup> H <sub>2</sub> -(CH <sub>2</sub> ) <sub>2</sub> -CH <sub>3</sub> ) <sub>2</sub>	1.94

In summary, the results of the manifold studies at that time showed that the preparation of a deuterated watch oil for industrial application provides the best perspectives for technical use of kinetic isotope effects of deuterium. Following a proposal of experts of the Verkehrshochschule Dresden, Germany, an aromatic ether alcohol was chosen due to excellent lubrication properties seen in earlier studies. To make this lubricant outstanding in age stability, too, deuterium was substituted for hydrogen in some  $\text{CH}_2$  groups.

As early as in 1962, a comprehensive patent was applied to protect the copyright for technical application of large deuterium isotope effects: 'Procedure to increase the chemical stability of hydrogen-containing substances', issued on 6 December 1965 [55]. The patent rights include all procedures to stabilise substances, their formulations, and mixtures in which during or after the preparation deuterium is completely or partially substituted for hydrogen. Moreover, supplementary stabilisers can be added. The substitution can also take place in solids near to their surface. The patent was issued in several other countries, too.

An additional patent was applied in 1968 and issued on 20 November 1970: 'Lubricants stabilised against aging' [56]. With this patent, the addition of several combined acting additives is protected.

Not to infringe the own copyright, a corresponding scientific publication was issued only in 1970: 'About the properties of a novel deuterated watch oil of the type ether-alcohol (Deuterosynth®)' [57]. In Figure 2, ageing of different watch oils is compared including the new one, indicating its predominance.

The high quality of Deuterosynth was certified by the Swiss Watch Test Institute Neuchâtel. Due to its excellent age stability, the oil was accepted by the corresponding industry. An oil-refining company started with the production of the oil in 1980, after a long-time ageing control (by 1-year intensive shaking under oxygen atmosphere at  $40^\circ\text{C}$ ) had not shown any change in colour and viscosity.

To the surprise of the Leipzig group, a US American patent was issued in 1973 about a 'Deuterated lubrication oil' [58], 8 years after the international acceptance of the claims of the East-German patent [55].

As a result of extended application research in the Leipzig Institute of Stable Isotopes on deuterium isotope effects, several further patents were issued, such as:

- procedure to enhance the stability of photographic materials [59] proclaiming a decrease in fog tendency of conventional photoreceptor coats of film and paper materials by a factor of 2.5 after final adjustment of their residual moisture with  $^2\text{H}_2\text{O}$ ;
- deuterated liquid scintillation mixtures [60] proclaiming an increase in the effectivity of a scintillation cocktail by about 90% after deuteration of the solvent and the solubilisers;
- procedure to improve nuclear spin tomographical measurements of biological objects [61], especially tracing of the kinetics of drug metabolisms *in vivo* proclaiming the measurement of the non-deuterated compound in turn with the same compound in deuterated form.

In the 1980s, the Leipzig group broke up. As expected, maybe partly inspired by the publications of the Leipzig group, other groups also worked in this field. It is supposed, however, that already from the beginning, business companies in Germany and in other countries arranged corresponding studies and mainly prevented publications in scientific journals.

As a consequence, further technical use of deuteration can be followed practically only via the patent literature. Some of the patents were issued originally in the USA, such as:

- deuterium treatment of semiconductor devices [62] proclaiming an annealing process with deuterium at superatmospheric pressures to improve reduction of the effects of hot carrier stress during device operation;

- deuterium sintering with rapid quenching [63] proclaiming the increase in deuterium concentration near the interface for diffusion and influencing the Si–H by Si–D bonds;
- application of deuterium oxide in producing silicon-containing and metal compounds [64] proclaiming an optical compound material for use in optical devices in the wavelength range between 1.0 and 1.8  $\mu\text{m}$ , wherein substantially most OH bonds are substituted by OD bonds;
- method and apparatus for fabricating optical fibre using deuterium exposure [65] proclaiming fibreglass having reduced ageing or hydrogen ageing loss over the life of the fibre.

As expected, some patent applications are aimed at using deuterated compounds in medicine, too. An interesting ‘multi’-patent application was claimed only recently. Altogether, Czarnik has trademarked as many as 235 ‘deuterium-enriched’ substances in their ‘pharmaceutically acceptable salt forms’, mostly antibiotics. For example, one of the patents describes ‘deuterium-enriched ranolazine, pharmaceutically acceptable salt forms thereof, pharmaceutical compositions containing the same, and methods of treating using the same’ [66]. Another one reads ‘deuterium-enriched fluticasone propionate, pharmaceutical compositions containing the same, and methods of using the same’ [67].

In 2010, another patent was published aiming at the ‘use of deuterium oxide for treating viral diseases of the respiratory tract’. The inventor argues that  $^2\text{H}_2\text{O}$  alone or in combination with conventional drugs sprayed on the mucosa membrane has a positive effect due to the  $^2\text{H}$ -bridges for  $^1\text{H}$ -bridges of the water. Thus, the replication of pathogenic cells on the mucosa should be inhibited [68].

Surprisingly, a curious-seeming idea was published relatively early, in fact not as a patent. Katz and Crespi [69] argued that living organisms of unusual isotopic composition can be used for NMR studies. Fully deuterated organisms were seen as an artefact. ‘They may differ significantly in morphology, cytochemistry, and biosynthetic capacity but basically they must be the same organism’.

At least, some further independent scientific publications characterise the progress of the art over the past 30 years.

Deuterium isotope effects on lymphoid tissues and humoral antibody responses in mice were studied in a Swiss hospital and in the Brookhaven National Laboratory in the 1970s [70]. Marked atrophy of lymphoid tissues after exposure of mice to various doses of heavy water and structural changes due to the deuterium-induced suppression of primary and secondary tetanus antitoxin responses were described.

A recent review from India refers to the investigation of superior thermal stability of oral polio vaccine prepared in heavy water as well as to the stabilisation of compounds with deuterium for their application in various high-technology areas, mainly in microelectronics [71]:

- Deuterated polymethyl methacrylate and polystyrene used as optical fibres show a reduction of intensity loss by 6 to 10 times.
- Silica-based optical fibres treated with gaseous deuterium have an improved ageing resistance.
- Deuterated polymers show a much higher optical recording density of compact and laser discs by a factor of 1000.
- Deuterated polycarbonates have much better mechanical and chemical resistance properties.
- Deuterated metal oxide gates produce a very thin layer of oxide film with reduced stress-induced leakage current and thereby enhancing the life of the components.
- Deuterated lubricants and deuterated solvents as mentioned above.

Unfortunately, no references are given to the claims which may be removed mainly from patents. According to the authors, India is starting with corresponding research and application.

## 5. Conclusion

Since the late 1950s, deuterium isotope effects have been used or have been proposed to be used mainly in two application areas: pharmaceutical and high-technology products.

Starting with first investigations by Katz *et al.* [72], many clinical–pharmacological investigations have been specified. In 1973, a first comprehensive summary by Blake *et al.* [73] covered already more than 200 references using the term ‘deuterated drug’. The deuterated compounds provide a basis to follow kinetics and mechanisms of drug metabolism *in vivo*. Isotope effects are sometimes used to drive a compound to be metabolised in the desired direction. Due to intended commercial use, in case of *in vivo* application of deuterated drugs in the clinical field of diagnosis and therapy, the names of the compounds are mostly encoded, if published at all, as seen only recently [74,75].

An interesting insight into current activities of pharmaceutical companies to market deuterated drugs is given by an article published in *Nature* [76]. The company Concert Pharmaceuticals (Lexington, MA, USA) has successfully finished the phase 1 clinical trial of a deuterated version of the antidepressant paroxetine testing 94 women. The company Auspex Pharmaceuticals (Vista, CA, USA) also announced phase 1 clinical trial results with an antidepressant, in that case with a deuterated version of venlafaxine. Both results show longer effective times of the deuterated drug in the body. In the meantime, the Concert company has decided not to develop deuterated paroxetine ‘further for now’ and is focusing instead on another deuterated drug, an HIV protease inhibitor, expecting it to be a bigger seller. It is remarkable that these activities have their roots more than 40 years ago when in the 1960s first corresponding results were published [31,39,40,44,51].

Work on the other field of the use of large kinetic deuterium isotope effects – application in high-technology areas – was started in Leipzig, also in the late 1950s [43,55] and successfully continued there. Unfortunately, at that time, all corresponding papers were published in German language [77]. This review may contribute to bridge a corresponding gap.

In future, to the author’s opinion, good prospects of the practical application of large deuterium isotope effects are given in medicine and in quality improvement of costly technical products, for instance, in space exploration techniques.

Let us watch the development and do the next review in 10 years.

## References

- [1] P. Krumbiegel, *Isotopieeffekte* (Akademie-Verlag, Berlin, Pergamon Press, Oxford, Vieweg + Sohn, Braunschweig, 1970), p. 11 (in German).
- [2] J. Bigeleisen and M. Goeppert-Mayer, Calculation of Equilibrium Constants for Isotopic Exchange Reaction, *J. Phys. Chem.* **15**, 261 (1947).
- [3] K.B. Wiberg, Kinetic Deuterium Isotope Effects, *Chem. Rev.* **55**, 713 (1955).
- [4] J. Bigeleisen and M. Wolfsberg, Theoretical and Experimental Aspects of Isotope Effects in Chemical Kinetics, *Adv. Chem. Phys.* **1**, 15 (1958).
- [5] L. Melander, *Isotope Effects on Reaction Rates* (Ronald Press Co., New York, 1960).
- [6] G. Vojta, Theorie der thermodynamischen Isotopieeffekte, in *Lehrbuch der Kernphysik*, edited by G. Hertz (B.G. Teubner, Leipzig, 1962), Vol. III, pp. 88–116.
- [7] V.G. Plekhanow, *Isotope Effect Application in Solids* (Springer, Berlin, 2004).
- [8] A. Kohen and H.H. Limbach, *Isotope Effects in Chemistry and Biology* (CRP Press, Boca Raton, London, New York, 2006).
- [9] M. Wolfsberg, W.A. van Hook, P. Paneth, and L.P.N. Rebelo, *Isotope Effects in the Chemical, Geological, and Bio Sciences* (Springer, Dordrecht, Heidelberg, London, New York, 2010).
- [10] H.B. Erlenmeyer, W. Schoenauer, and H. Sullman, Chemical and Biochemical Dehydrogenation of an Ethane- $\alpha$ -d, $\alpha$ -d-dicarboxylic Acid, *Helv. Chim. Acta* **19**, 1376 (1936).
- [11] J.F. Thomson, *Biological Effects of Deuterium* (The Macmillan Press, New York, 1963).

- [12] D. Rittenberg, The Influence of Nuclear Mass on Biological Systems, in *Isotopic and Cosmic Chemistry* (North Holland Publishing Co., Amsterdam, 1964), pp. 60–70.
- [13] A. Hughes, E.L. Bennet, and M. Calvin, Production of Sterility in Mice by Deuterium Oxide, *Proc. Natl Acad. Sci.* **45**, 581 (1959).
- [14] D. Wolf, H. Cohen, A. Mcshorer, I. Wassermann, and D. Samuel, The Effect of  $^{18}\text{O}$  on the Growth and Reproduction of Mice, in *Proceedings of the 3rd International Conference on Stable Isotopes*, Oak Brook (Academic Press, New York, 1979), pp. 353–360.
- [15] E. Borek and D. Rittenberg, Anomalous Growth of Microorganisms Produced by Changes in Isotopes in Their Environment, *Proc. Natl Acad. Sci.* **46**, 777 (1960).
- [16] C. Gregg, D. Ott, L. Deaven, N. Spielmann, K. Krowke, and D. Neubert, The Search for Biological Effects of  $^{13}\text{C}$  Enrichment in Developing Mammalian Systems, in *Proceedings of the 2nd International Conference on Stable Isotopes*, Oak Brook, 1975, pp. 61–70.
- [17] R. Medina, T. Ollerros, and H.-L. Schmidt, Isotope Effects on C- and N-Atoms as a Tool for the Elucidation of Enzyme-Catalysed Amide Hydrolysis, in *Proceedings of the 4th International Conference on Stable Isotopes*, Jülich (Elsevier, Amsterdam, 1981), pp. 77–82.
- [18] P. Krumbiegel, *Stable Isotope Pharmaceuticals for Clinical Research and Diagnosis* (G. Fischer, Jena, Stuttgart, New York, 1991), pp. 31–37.
- [19] P. Krumbiegel, Basic Principles in Clinical Use of Stable Isotope Pharmaceuticals, in *Principles of Radiopharmacology*, edited by H. Deckart and P.H. Cox (G. Fischer, Jena; M. Nyhoff, Den Haag, 1987), pp. 183–190.
- [20] C.K.C. Wang, T.E. Blue, and R. Gahbauer, A Neutronic Study of an Accelerator-Based Neutron Irradiation Facility for Boron Neutron Capture Therapy, *Nucl. Technol.* **84**, 93 (1989).
- [21] IAEA, *Assessment of Body Composition and Total Energy Expenditure in Humans Using Stable Isotope Techniques* (IAEA, Vienna, 2009).
- [22] TGL 14466 (DDR): *Stabilisotop markierte chemische Elemente und Verbindungen, Blatt 3: Anwendung in der Humanmedizin und Lebensmittelindustrie* (Fachbuchverlag, Leipzig, 1969).
- [23] Catalog Stohler/KOR, 'Stable Isotopes' (Cambridge, MA, 1985).
- [24] P.J.H. Jones and S.T. Leatherdale, Stable Isotopes in Clinical Research: Safety Reaffirmed, *Clin. Sci.* **80**, 277 (1991).
- [25] D.J. Kushner, A. Baker, and T.G. Dunstall, Pharmacological Uses and Perspectives of Heavy Water and Deuterated Compounds, *Can. J. Physiol. Pharmacol.* **77**, 79 (1999).
- [26] P. Krumbiegel, Berücksichtigung von Isotopieeffekten beim Arbeiten mit stabilen Isotopen in der organischen Chemie, *Isotopentechnik* **1**, 190 (1961).
- [27] A.I. Schatenstein, *Isotopenaustausch und Substitution des Wasserstoffs in organischen Verbindungen* (Übersetzung aus dem Russischen), (Deutscher Verlag der Wissenschaften, Berlin, 1963).
- [28] W.A. van Hook, in *Handbook of Nuclear Chemistry*, edited by A. Vertes, S. Nagy, and Z. Klencsar (Kluwer, Dordrecht, 2003), Vol. 5, p. 177.
- [29] A.B. Foster, Deuterium Isotope Effects in the Metabolism of Drugs and Xenobiotics: Implications for Drug Design, *Adv. Drug Res.* **14**, 1 (1985).
- [30] M.G. Horning, K.D. Haegele, K.R. Sommer, J. Nowlin, M. Stafford, and J.-P. Thenot, Metabolic Switching of Drug Pathways as a Consequence of Deuterium Substitution, in *Proceedings of the 2nd International Conference on Stable Isotopes*, Oak Brook, 1975, pp. 41–54.
- [31] M. Tanabe, D. Yasuda, S. LeValley, and C. Mitoma, The Pharmacologic Effect of Deuterium Substitution on 5-*n*-Butyl-5-Ethylbarbituric Acid, *Life Sci.* **8**, 1123 (1969).
- [32] R.I. Mazza and D.D. Denson, Deuteration of Anaesthetics, *Anaesthesiology* **51**, 101 (1979).
- [33] P.B. Farmer, A.B. Foster, M. Jarman, J.H. Kiburis, D.R. Newell, and D.J. Reed, Deuterium Isotope Effects in the Metabolism of Anti-Cancer Agents, in *Proceedings of the 3rd International Conference on Stable Isotopes*, Oak Brook, 1979, pp. 371–378.
- [34] G. Darland, H. Kropp, F.M. Kahan, R. Hajdu, R. Walker, and W.J.A. VandenHeuvel, The Metabolism of 2-Deutero-3-fluoro-D-alanine (DFA), in *Proceedings of the 2nd International Conference on Isotope Laboratory Compounds*, Kansas City (Elsevier, Amsterdam, 1986), pp. 277–281.
- [35] E. Larsen, Synthesis and Manufacturing Considerations for Deuterated Inhalation Anaesthetics, in *Proceedings of the 2nd International Conference on Isotope Laboratory Compounds*, Kansas City (Elsevier, Amsterdam, 1985), pp. 267–270.
- [36] W.A. Garland, The Use of Deuterated Analogs of Drugs as Medicinal Agents: Introduction and Report of Discussion, in *Proceedings of the 2nd International Conference on Isotope Laboratory Compounds*, Kansas City (Elsevier, Amsterdam, 1985), pp. 283–284.
- [37] A.B. Foster, Deuterium Isotope Effects in Studies of Drug Metabolism. Trends in Pharmacology, *Pharmacol. Sci.* **5**, 524 (1984).
- [38] H. Hübner, K. Kellner, P. Krumbiegel, and M. Mühlstädt, Über Lösungsmittel-Isotopieeffekte bei der Hydrolyse von para-substituierten Phenyl-dimethylcarbinylchloriden, in *Proceedings 3. Arbeitstagung über Stabile Isotope*, Leipzig, 1963, *Abhandl. Deutsche Akad. Wiss. zu Berlin* (Akademie-Verlag, Berlin, 1965), Vol. 7, pp. 679–685.
- [39] P. Krumbiegel and O. Serfas, Die Stabilisierung von Indocyaningrün in Injektionslösungen mit Hilfe von schwerem Wasser und Aminosäuren, *Arch. Pharm.* **299**, 432 (1966).

- [40] P. Krumbiegel and R. Schädel, Verfahren zur Herstellung stabilisierter wäßriger Lösungen von Indocyaninfarbstoffen, Patentschrift WP 43254 of DDR (issued 15 November 1965) and 244492A1 (issued 8 April 1987); Offenlegungsschrift 1469676 of Deutsches Patentamt (8 May 1969); Patentschrift 255.654 of Österreichisches Patentamt (10 July 1967); Patent Specification 1.048.419 of Great Britain (issued 18 March 1965); Brevet d'Invention of France 1.448.847 (issued 17 May 1965).
- [41] R. Wu, M.-M. Georgescu, F. Delpeyroux, S. Guillot, J. Balanant, K. Simpson, and R. Crainic, Thermostabilization of Live Virus Vaccines by Heavy Water (D<sub>2</sub>O), *Vaccine* **13**, 1058 (1995).
- [42] A. Liepins, Methods and Composition for the Treatment of Hypertension, US patent 5223269 (29 June 1993).
- [43] P. Krumbiegel, Isotopieeffekte bei Autoxydationen. 1. Mitt.: Kinetische Untersuchungen bei der katalytischen Oxydation von Xylofen durch Markierung mit Deuterium, *Isotopentechnik* (Berlin) **1**, 73 (1960/61).
- [44] J. Mühlenpfordt, Veredelung industrieller Erzeugnisse durch den Ersatz von Wasserstoff durch Deuterium. 1. Mitt., *Isotopenpraxis* **2**, 119 (1966).
- [45] H. Hübner, P. Krumbiegel, G. Möbius, S. Rummel, and M. Wahren, Veredelung industrieller Erzeugnisse durch den Ersatz von Wasserstoff durch Deuterium. 2. Mitt.: Deuteriumsubstitution als neue Methode der Schmierstoff-Veredelung, *Schmierstoffe und Schmierstechnik* **9**, 30 (1966).
- [46] P. Krumbiegel, H. Hübner, and M. Wahren, Veredelung industrieller Erzeugnisse durch den Ersatz von Wasserstoff durch Deuterium. 3. Mitt.: Kinetische Isotopieeffekte des Wasserstoffs bei der Autoxydation von Bernsteinsäuredibenzylester als Modellschmierstoff, *Isotopenpraxis* **2**, 185 (1966).
- [47] S. Rummel, Wasserstoff-Isotopieeffekt bei Autoxydationen organischer Verbindungen in Abhängigkeit von der Struktur, der Deuterium-Häufigkeit und der Deuterium-Stellung, Dissertation, Universität Leipzig, 1968.
- [48] S. Rummel, H. Hübner, and P. Krumbiegel, Isotopieeffekte bei Autoxydationen. 3. Mitt.: Sekundärer Beta-Deuterium-Isotopieeffekt in der thermischen Zersetzung des (D<sub>12</sub>)Azo-bis-isobutyronitrils, *Z. Chemie* **7**, 351 (1967).
- [49] S. Rummel, H. Hübner, and P. Krumbiegel, Isotopieeffekte bei Autoxydationen. 4. Mitt.: Effektivität der Radikalbildung aus (D<sub>12</sub>)Azo-bis-isobutyronitril. Wasserstoff-Isotopieeffekt, *Z. Chemie* **7**, 392 (1967).
- [50] K. Schmidt, S. Rummel, G. Tamme, P. Krumbiegel, and H. Hübner, Registrierende Meßanordnung für Volumenänderungen bei chemischen Reaktionen unter Beteiligung von Gasen, *Chem. Technik* **20**, 357 (1968).
- [51] P. Krumbiegel, Große reaktionskinetische Wasserstoff-Deuterium-Isotopieeffekte unter dem Gesichtspunkt ihrer Nutzung, *Z. Chemie* **8**, 328 (1968).
- [52] S. Rummel, P. Krumbiegel, and H. Hübner, Modelluntersuchungen zur oxydativen und thermischen Stabilität von Dicarbonsäureestern, *J. Prakt. Chem.* **311**, 677 (1969).
- [53] U. Weisflog, P. Krumbiegel, and H. Hübner, Isotopieeffekte bei Autoxydationen. 7. Mitt.: Wasserstoff-Bruttoisotopieeffekt bei der Autoxydation von Benzyläthern, *Isotopenpraxis* **6**, 285 (1970).
- [54] S. Rummel, P. Krumbiegel, and H. Hübner, Isotopieeffekte bei Autoxydationen. 5. Mitt.: Über die Abhängigkeit der Größe des kinetischen Isotopieeffektes von der relativen Deuterium-Häufigkeit, *Isotopenpraxis* **6**, 134 (1970).
- [55] J. Mühlenpfordt, H. Hübner, K. Wetzel, M. Wahren, and P. Krumbiegel, Verfahren zur Erhöhung der chemischen Beständigkeit wasserstoffhaltiger Substanzen, Patentschrift WP 41827 of DDR (issued 6 December 1965) and corresponding patents in other countries.
- [56] H. Hübner, P. Krumbiegel, and S. Rummel, Gegen Alterung stabilisierte Schmieröle, Patentschrift WP 76291 of DDR (issued 20 August 1970).
- [57] H. Reith, P. Krumbiegel, S. Rummel, M. Wahren, H. Hübner, and K. Kuhn, Über die Eigenschaften eines neuartigen Uhrenöls vom Typ Ätheralkohol ('Deuterosynth®'), *Uhren und Schmuck* **7**, 146 (1970).
- [58] J.G. Atkinson and M.O. Luke, Deuterated lubrication oil, US patent 3.746.634 (issued 17 July 1973).
- [59] P. Krumbiegel and J. von Grossmann, Verfahren zur Erhöhung der Haltbarkeit photographischer Materialien, Patentschrift WP 46632 of DDR (issued 20 March 1966).
- [60] P. Krumbiegel and H. Schmidt, Flüssigkeitsszintillationsmischung, Patentschrift WP 79537 of DDR (issued 05 February 1971).
- [61] P. Krumbiegel, P. Junghans, and S. Gursky, Verfahren zur Verbesserung kernspintomographischer Messungen biologischer Objekte, Patentschrift WP 225.789 of DDR (issued 07 August 1985).
- [62] J.W. Lyding, K. Hess, and J. Lee, Deuterium Treatment of Semiconductor Devices, US patent 2003/0219950 A1 (issued 24 July 2002).
- [63] R.M. Wallace and K.C. Harvey, Deuterium Sintering with Rapid Quenching, US patent 6071751 (issued 06 June 2000).
- [64] Z. Zhang, G. Xiao, G. Pan, P. Zhang, and M. Zhou, Application of Deuterium Oxide in Producing Silicon Containing and Metal Compounds, US patent 2001/0047665 A1 (issued 07 May 2001).
- [65] K.H. Chang, D. Kalish and T.J. Miller, Method and Apparatus for Fabricating Optical Fiber Using Deuterium Exposure, US patent 2004/0011081 A1 (issued 15 July 2003).
- [66] A.W. Czarnik, Deuterium-enriched Ranolazine, UA patent 51425212 (issued 19 March 2009).
- [67] A.W. Czarnik, Deuterium-enriched Fluticasone Propionate, US Patent 20080318915 (issued 25 December 2008).
- [68] T. Bayerl, Use of Deuterium Oxide for Treating Viral Diseases of the Respiratory Tract, WIPO Patent WO/2010/079420 (issued 15 July 2010).
- [69] J.J. Katz and H.L. Crespi, Deuterated Organisms: Cultivation and Uses, *Science* **151**, 1187 (1966).
- [70] J.A. Laissue and R.D. Stoner, Deuterium Isotope Effects on Lymphoid Tissues and Humoral Antibody Responses in Mice, *Virchows Arch. A Path. Anat. Histol.* **383**, 149 (1979).
- [71] M. Bhaskaran and R. Prakash, Alternate Applications of Heavy Water in Biological and Technological Fields, in *Proceedings of the 16<sup>th</sup> Annual Conference of the Indian Nuclear Society*, November 2005.

- [72] J.J. Katz, H.L. Crespi, R.J. Hasterlik, J.F. Thomson, and A.J. Finkel, Some Observations on Biological Effects of Deuteration with Special Reference to Effect on Neoplastic Processes, *J. Natl Cancer Inst.* **18**, 64 (1957).
- [73] M.I. Blake, H.L. Crespi, and J.J. Katz, Studies with Deuterated Drugs, *J. Pharm. Sci.* **64**, 367 (1975).
- [74] V. Uttamsingh, *CTP-347, a Deuterated Paroxetine Analog Exhibits Reduced Mechanism-based Inactivation of CYP 206 in Healthy Women*, 38th College of Clinical Pharmacology, 2009.
- [75] S. Harbeson and R. Tung, Chemistry for Drug Development: Deuterium Modification, *Drug Discov. Dev. Mag.* **13**, 22 (2010).
- [76] K. Sanderson, Big Interest in Heavy Drugs, *Nature* **458**, 269 (2009).
- [77] P. Krumbiegel, Stabilisotope Arzneimittel, Berichte des Zentralinstituts für Isotopen- und Strahlenforschung Leipzig, *ZfI-Mitteilungen* **118**, 41 (1986).